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An electrochemical biosensor for the rapid detection of erythropoietin in blood

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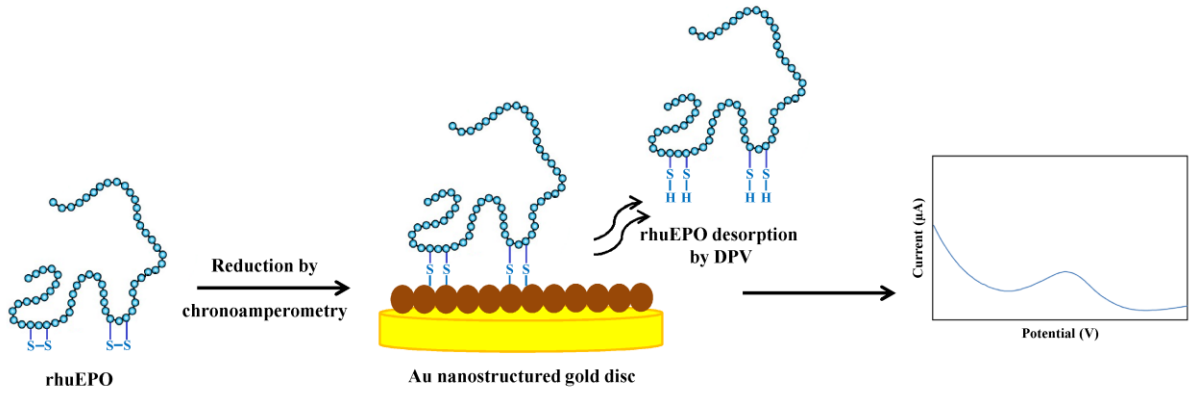
Abstract

A label free electrochemical detection method for the rapid detection of recombinant human erythropoietin (rhuEPO) has been developed. In this method, we modified the rhuEPO structure for its direct sensing without using a complex signal amplification strategy. The protein was selectively extracted from blood plasma sample using target-specific magnetic beads. After releasing rhuEPO from the magnetic beads, its disulfide bonds were electrochemically reduced and the protein was spontaneously assembled onto a nanostructured gold electrode via Au-S bonds formation. For electrochemical quantification, the reduced protein was desorbed from the electrode surface using differential pulse voltammetry (DPV). The desorption current was proportional to the concentration of rhuEPO in the range 1 pM – 1000 pM. By cross-validating against ELISA, we found a 104.85 ± 3.35 % agreement between the results obtained using the electrochemical biosensor and ELISA. Therefore the developed method has a strong potential for the sensitive detection of rhuEPO doping in sports as well as its rapid screening and pathology labs.

[1] [1]W.A. Hassanain and [1]A. Sivanesan contributed equally to the manuscript.

Graphical abstract



Key words

Recombinant human erythropoietin, Disulfide bond reduction, Electrochemical biosensing, Nanostructured gold electrode, Chronoamperometry, DPV.

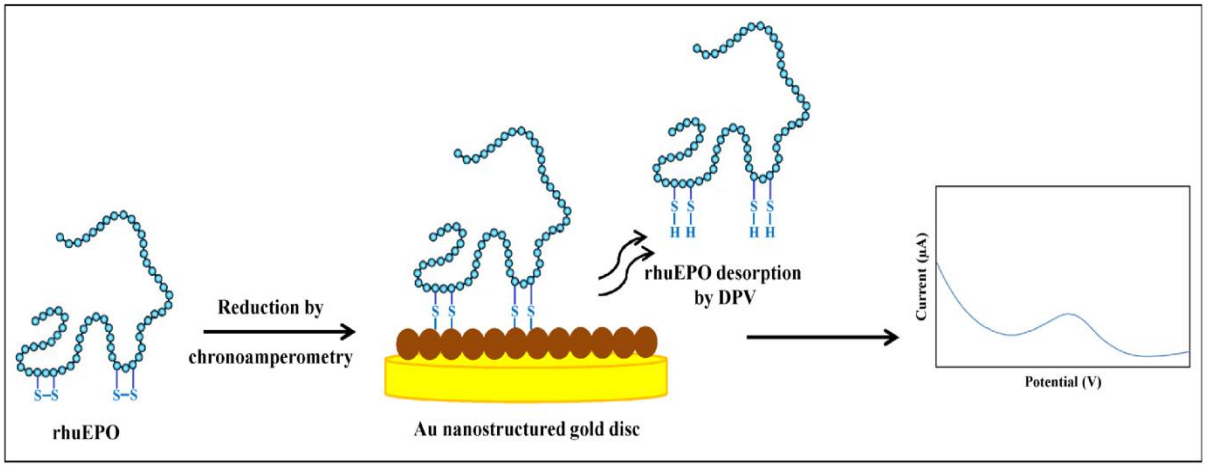
1. Introduction

Erythropoietin (EPO) is a naturally occurring hormone that is used for the regulation of erythropoiesis and as a signalling protein for the production of red blood cells in bone marrow [1]. Recombinant human EPO (rhuEPO) is used for the treatment cancer patients suffering from associated anaemia [2]. In the sports industry rhuEPO is doped by athletes to increase their oxygen capacity and enhance their aerobic performance [3]. The widespread doping of rhuEPO has prompted the World Anti-Doping Agency (WADA) to ban its use in international sports and sponsor significant research on sensitive detection methods of the protein in blood and urine. Similar to other protein biomarkers, the screening of rhuEPO in biological fluids requires accurate and sensitive and selective detection methods. The current analysis methods for rhuEPO detection include isoelectric focusing, double blotting, HPLC-

MS and MALDI-TOF techniques [4]. These techniques require lengthy analysis time, expensive equipment, and skilled operators to perform complex screening procedures.

Electrochemical methods are among the simple and non-expensive techniques for protein analysis [5]. Electrochemical sensors are characterised by their rapid response, small sample volume requirement, cost-effectiveness and potential for miniaturization within portable analytical platforms [6-8]. The direct electrochemical detection of proteins suffer from inadequate sensitivity and selectivity towards the target biomolecule [9, 10]. Therefore, the electrochemical biosensing of proteins is usually limited to those having non-protein redox centres allowing for the reversible electron transfer between the electrode and the biomolecule [5]. To improve the sensitivity of the electrochemical biosensors, derivatization reagents have been employed to lower the detection limit of anlaytes to the nanomolar range [11]. In addition, nanostructured electrodes have been utilised to enhance the sensitivity of the electrochemical detection of analytes [12, 13].

In this work, we present a proof of concept for a rapid and sensitive label-free electrochemical determination of rhuEPO in blood plasma down to pico molar concentrations. To selectively extract rhuEPO from blood plasma, an anti-rhuEPO antibody was conjugated to amine derivatized and silanized iron oxide magnetic beads. After binding the target protein, the pH of the beads was modified to release the extracted protein within 5 minutes. For direct detection, the protein disulfide bond structure was reduced by chronoamperometry on a nanostructured gold electrode to assemble the reduced protein onto the electrode surface via Au-S bond formation. The protein was then electrochemically desorbed by differential pulse voltammetry (DPV) in 1 minute, and the developing current is utilized for the quantification of the protein (Scheme 1). The new direct electrochemical biosensing of rhuEPO was cross validated against enzyme linked immunosorbent assay (ELISA).



Scheme 1. A schematic representation of the novel electrochemical biosensing of rhuEPO

2. Experimental

2. 1. Chemicals and materials

Amine derivatized and silanized magnetic beads with a particle size of 1-4 μm were purchased from Thermo Fisher Scientific (Au). Recombinant human EPO international standard (rhuEPO, 3rd international reference preparation) was sourced from the national institute for biological standards and control (UK). Anti-erythropoietin antibody (7D3) was purchased from MAIIA Diagnostics (SWE). Human EPO ELISA kits were purchased from Abcam (Au). Horse plasma samples were donated by Dr. Rohan Steel, Project Leader, Biological Research Unit, Racing Analytical Services Ltd, Melbourne (Au). The samples were collected and shipped to Queensland University of Technology (QUT) under the Melbourne lab protocols, ethical clearances and arrangements. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), phosphate buffered saline (PBS), glycine, potassium hydroxide, gold (III) chloride trihydrate ($HAuCl_4$ .3 H_2O) perchloric acid ($HClO_4$) and protein low bind eppendorf tubes were purchased from Sigma Aldrich (Au). Gravity flow size-exclusion columns (illustra NAP-5 (17-0853-01)) were obtained from GE Healthcare Life Sciences (Au). Deionized water (ultrapure Millipore,

18.2 MΩ cm@25 °C) was used in all preparations. The glassware and gold discs were cleaned with piranha solution (3:1, 98% H_2SO_4: 30% H_2O_2) and thoroughly rinsed with deionised water prior to use.

2. 2. Instruments

The electrochemical measurements were carried out using Autolab PGSTAT204 potentiostat (Metrohm Autolab, NL), equipped with NOVA 1.10.5 as operating software and a standard three electrode cell. Polycrystalline gold disc (geometric area = 0.502 cm^2) and platinum wire were used as working and counter electrodes, respectively. Dry leakless Ag/AgCl/saturated KCl (world precision instruments, USA) was used as a reference electrode. For ELISA screening, the optical density was measured at 450 nm using a multimode reader GloMax explorer (Promega, USA).

2. 3. Preparation of rhuEPO standard solutions

A series of standard solutions of rhuEPO in the concentration range of 1 pM – 1000 pM were prepared by serial dilution in 1x phosphate buffered saline buffer (PBS) (pH 7.4).

2. 4. Preparation of gold nanostructured electrode for DPV detection

Nanostructured gold electrodes were prepared as described by Sivanesan et al [14]. Briefly, flat gold disc electrodes were mirror-polished with alumina slurries of decreasing particle sizes (0.5 µm, 0.05 µm and 0.02 µm), immersed in deionised water and subsequently sonicated in aqueous ultrasonic bath for 15 minutes. The electrodes were then immersed in piranha solution [H_2SO_4 (98%): H_2O_2 (30%), 3:1 v/v] for 10 minutes and rinsed with deionised water. The gold electrode was utilised as a working electrode in a three electrode cell that is filled with 4 mM $HAuCl_4$ in 0.1 M $HClO_4$ and purged with argon gas for 30

minutes to remove oxygen gas from the solution. A negative potential of -0.08 V was applied for 15 minutes to electrochemically deposit gold nanostructures onto the gold disc. The gold nanostructured electrode was then removed from the cell, washed with deionised water, dried under nitrogen stream and stored for future use.

2. 5. Synthesis of antibody-conjugated magnetic beads

In our work, 100 µL of 0.2 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) solution was mixed with 100 µL of 0.1 M N-hydroxysuccinimide (NHS) solution in a low bind eppendorf tube and gently vortexed for 2 minutes. This was followed by the addition of 100 µL of the amine derivatized and silanized iron oxide magnetic beads. Finally, 10 µL of anti rhuEPO antibody (0.1 mg/mL) were added to the mixture. The resulting mixture was stored at 4 ºC for 4 hours to allow for the complete conjugation of the antibody to the magnetic beads. The conjugated beads were magnetically separated from the solution, re-suspended in 100 µL PBS (pH 7.4) and stored at 4 ºC for future use.

2. 6. Selective extraction of rhuEPO

The antibody-conjugated beads were used to selectively extract rhuEPO from blood plasma samples. Horse blood plasma was spiked with 250 pM of rhuEPO. This was followed by mixing 100 µL aliquot of the spiked plasma sample with 100 µL of the antibody-conjugated magnetic beads and left to stand for 15 minutes at room temperature with occasional shacking. The magnetic beads were collected from the plasma matrix using an external magnet and washed 5 times, to remove any weakly adsorbed interfering molecules (each time with 100 µL of 1x PBS buffer, pH 7.4).

To release the captured rhuEPO from the extractor beads, they were re-constituted into 100 µL of 0.1 M glycine.KOH buffer (pH 11) for 5 minutes at room temperature. The released

rhuEPO solution was loaded onto a size exclusion column to remove the glycine buffer. The purified protein was eluted using 500 µL of 0.1 M KOH.

2. 7. Electrochemical reduction and quantification of rhuEPO

For the electrochemical quantification of the rhuEPO, 50 µL aliquots of standard rhuEPO solutions in the concentration range 1 pM - 1000 pM were loaded onto nanostructured gold electrodes and reduced and assembled by chronoamperometry using a potential of -1 V for 30 minutes. The reduced protein was electrochemically desorbed from the electrode surface by DPV where the potential was swept between -1 V to -1.4 V at step of -0.005 V and a scan rate of 0.01 V/s. The pulse amplitude and width were set at 50 mV and 50 ms, respectively. The magnitude of the resulting desorption current at -1.19 V was plotted against the protein concentration to construct a calibration plot.

For the quantification of rhuEPO in blood plasma, 50 µL aliquot of the eluted rhuEPO, in section 2.6, was loaded onto the working nanostructured gold electrode of the electrochemical cell and dried under gentle stream of nitrogen gas (Figure S1). KOH was added as an electrolyte solution, and chronoamperometry followed by DPV measurements were carried out.

2. 8. ELISA cross-validation

To cross validate the DPV quantification of rhuEPO, an aliquot of the eluted protein in section 2.6 was screened using a human EPO ELISA kit. An ELISA calibration plot was constructed in the concentration range 10.7 – 333.2 pg/mL, where the average optical density (OD) at 450 nm was plotted against the concentration of the protein standards (Figure S2).

3. Results and discussion

3. 1. Selective extraction of rhuEPO from blood plasma

Direct electrochemical biosensing of biomolecules requires their selective isolation from the complex biological matrix prior to measurements [15, 16]. For this purpose, we developed rhuEPO extractor beads by conjugating the C terminal of an anti-rhuEPO antibody to the amine-terminals of silanized magnetic beads using the EDC coupling reaction [17, 18]. The EDC/NHS coupling was added to the amine derivatized and silanized iron oxide magnetic beads to activate the HN2 groups on the surface of the beads. The antibody was then added to the mixture, where the EDC/NHS reagents activated the COOH groups at the C terminal of the antibody. The activated NH_2 and COOH groups of the magnetic beads and the antibody respectively, react to form amide bonds that attach the antibody to the bead [17].

The antibody-functionalized beads were used to selectively bind and extract rhuEPO from blood plasma. By changing the pH environment of the beads, the weak coulombic and van der waals bonds between the antibody and the bound protein molecules were easily broken and the isolated rhuEPO molecules retrieved [19-22].

3. 2. Electrochemical reduction and assembly of rhuEPO on nanostructured gold electrode

The average range of EPO blood concentration in healthy humans is 0.92 – 3.7 pM [23]. Therefore, an electrochemical biosensor of rhuEPO should have a detection limit within the pico molar concentration range. We first attempted the direct electrochemical detection of 10 pM rhuEPO by DPV using a flat gold electrode. As indicated by Figure 1 (blue line), no characteristic desorption current was observed. Therefore, we repeated the DPV measurement using a nanostructured gold electrode [24, 25]. However, no characteristic desorption current was observed for rhuEPO (Figure 1, red line).

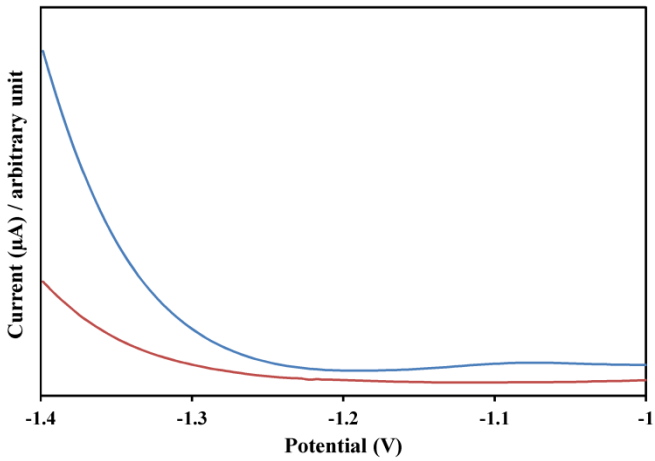


Figure 1: DPV of rhuEPO on a polished gold electrode (blue line) and on a nanostructured gold electrode (red line).

Another approach to enhance the electrochemical detection of analytes is to form covalent bonds between the target analyte and the nanostructured electrode [26]. Therefore, we used chronoamperometry to electrochemically reduce and assemble the protein onto the nanostructured gold electrode surface. The electrochemical reduction cleaves the disulfide bond between the cysteine residues of the protein and generates free sulfhydryl (SH) terminal groups that spontaneously form Au-S bonds with the electrode surface [27]. The assembled protein molecules were then desorbed from the electrode by DPV, where a characteristic desorption current was observed at -1.19 V as depicted by Figure 2 (blue line).

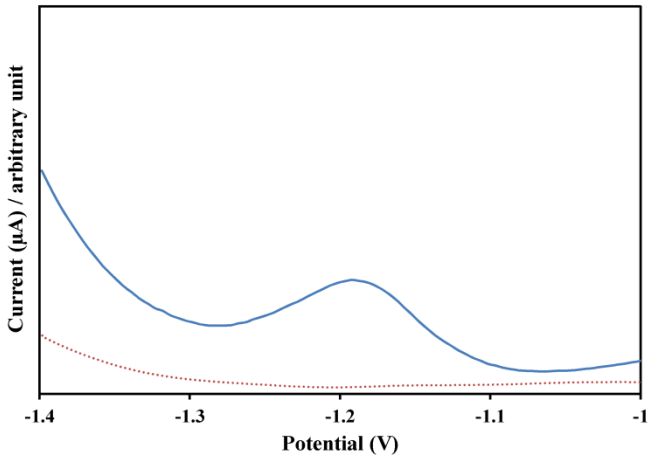


Figure 2: DPV of reduced EPO on a nanostructured gold electrode, the red dotted line denoted the blank nanostructured electrode.

The effect of reduction time on the DPV measurement was investigated where chronoamperometry was carried at 10, 20, 25, 30, 35 minutes respectively and the DPV current measured after the reduction. As indicated by Figure S3, the DPV current was maximum after 30 minutes of chronoamperometric reduction. Therefore, to allow for a sensitive detection of the protein, the chronoamperometry was lasted for 30 minutes before the DPV measurement.

The electrochemical desorption current of reduced rhuEPO was found to proportionally increase with the protein concentration in the range 1–1000 pM and follow a polynomial relationship (Figure 3a, b). The inset in Figure 3b depicts the linear relationship between the DPV current and the concentration of rhuEPO in the concentration range of 1 to 250 pM. The regression equation for the linear relationship was found to be $y = 0.002x + 0.0979$ (R^2 =0.983). Using this regression equation, the 95% confidence intervals for the slope and the intercept were found to be 0.0008 and 0.109, respectively. The limit of detection was found to be 1 pM.

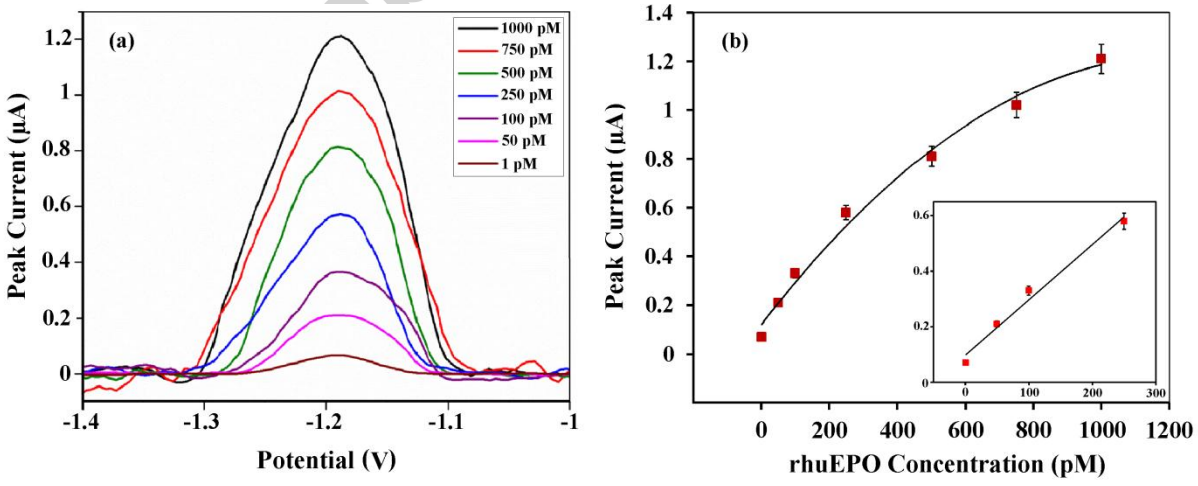


Figure 3: **(a)** DPV of reduced rhuEPO on nanostructured gold electrode in the concentration range 1 – 1000 pM, **(b)** corresponding calibration curve in the same concentration range. The inset depicts the linear relationship in the concentration range 1-250 pM.

3. 3. DPV quantification of rhuEPO in blood plasma and cross validation by ELISA

To utilize the developed electrochemical method methodology for the biosensing of rhuEPO in blood plasma, the protein was extracted using the functionalized beads, reduced by chronoamperometry on a nanostructured gold electrode and quantified by DPV. The average concentration of rhuEPO in the plasma sample was found to be 221 ± 0.44 pM (n=3) by DPV. The standard deviation within the three measurements was 1.05.

For a cross validation purpose, another aliquot of the same extract was screened by ELISA. The ELISA screening test confirmed the presence of rhuEPO in the plasma sample at an average concentration of 211 ± 7.17 pM (n=3). Therefor the average agreement between the DPV and the ELISA measurements was 104.85 ± 3.35 %.

Zhang et al. recently demonstrated a method for the electrochemical detection of rhuEPO down to 0.016 fM [28]. The authors used a signal amplification approach where a glassy carbon electrode modified with a sandwich-like nano-Au/ZnO sol-gel/nano-Au compound membrane was manufactured and used for the detection of the protein by monitoring the change in the electrode response. The dynamic range of this method was 0.016 fM – 0.016 nM and the limit of detection was 0.003 fM. Considering the complex procedures and the long preparation time of the electrode (~12 hours), our developed direct electrochemical biosensing method is simple, rapid and can be used for monitoring rhuEPO doping and therapeutic use [29]. In addition, the nanostructured gold electrode used in this work can be easily recycled by removing the contaminated gold nanostructures using a simple mechanical

etching procedure, followed by an electrochemical deposition of new gold nanostructures within 15 minutes.

4. Conclusion

In this work, we presented a simple electrochemical method for the rapid and sensitive detection of proteins, as demonstrated by rhuEPO, in blood plasma. We developed target-specific antibody-conjugated magnetic beads for the selective extraction of the protein from blood plasma within 15 minutes. For its sensitive electrochemical biosensing, the extracted protein was electrochemically reduced and assembled by chronoamperometry on a nanostructured gold electrode. The assembled reduced protein was then desorbed from the electrode surface by DPV to give an electrochemical desorption current that is proportional to the concentration of rhuEPO within the range of 1-1000 pM which is useful for monitoring rhuEPO doping and therapeutic use. The DPV determination of rhuEPO was cross validated against ELISA where the agreement between the 2 measurements was 104.7%. The developed electrochemical approach method has a strong potential for the rapid, sensitive and cost effective determination of other proteins in molecular diagnosis applications.

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References

[1] W. Jelkmann, Regulation of erythropoietin production, J. Physiol. 589 (2011) 1251-1258.

[2] A. Engert, Recombinant human erythropoietin in oncology: current status and further developments, Annals of Oncology 16 (2005) 1584–1595.

[3] S. Elliott, Erythropoiesis-stimulating agents and other methods to enhance oxygen transport, British Journal of Pharmacology 154 (2008) 529–541

[4] J. R. Delanghe, M. Bollen, M. Beullens, Testing for recombinant erythropoietin. Am. J. Hematol. 83 (2008) 237-41

[5] E. Paleček, J. Tkáč, M. Bartošík, T. Bertók, V. Ostatná, J. Paleček, Electrochemistry of Nonconjugated Proteins and Glycoproteins. Toward Sensors for Biomedicine and Glycomics, Chem. Rev. 115 (2015) 2045-2108.

[6] E.B. Bahadıra, M.K. Sezgintürk, Electrochemical biosensors for hormone analyses, Biosens. Bioelectron. 68 (2015) 62-71.

[7] A.L. Furst, N.B. Muren, M.G. Hill, J.K. Barton, Label-free electrochemical detection of human methyltransferase from tumors, PNAS 111 (2014) 14985-14989.

[8] W. Deng, B. Xu, H. Hu, J. Li, W. Hu, S. Song, Z. Feng, C. Fan, Diagnosis of schistosomiasis japonica with interfacial co-assembly-based multi-channel electrochemical immunosensor arrays, Sci. Rep. 3 (2013) DOI: 10.1038/srep01789.

[9] E.J. Nam, E.J. Kim, A.W. Wark, S. Rho, H. Kim, H.J. Lee, Highly sensitive electrochemical detection of proteins using aptamer-coated gold nanoparticles and surface enzyme reactions, Analyst 137 (2012) 2011-2016.

[10] K. Yang, C. Zhang, Improved Sensitivity for the Electrochemical Biosensor with an Adjunct Probe, Anal. Chem. 82 (2010) 9500-9505.

[11] T. Sierra, A.G. Crevillen, A. Escarpa, Derivatization agents for electrochemical detection in amino acid, peptide and protein separations: The hidden electrochemistry?, Electrophoresis 38 (2017) 2695-2703.

[12] Y.E. Choi, J.W. Kwak, J.W. Park, Nanotechnology for Early Cancer Detection, Sensors 10 (2010) 428-455.

[13] P. Jolly, M.R. Batistuti, A. Miodek, P. Zhurauski, M. Mulato, M.A. Lindsay, P. Estrela, Highly sensitive dual mode electrochemical platform for microRNA detection, Sci. Rep. 6 (2016) Doi:10.1038/srep36719.

[14] A. Sivanesan, W. Adamkiewicz, G. Kalaivani, A. Kamińska, J. Waluk, R. Hołyst, E.L. Izake, Electrochemical pathway for the quantification of SERS enhancement factor, Electrochem. Comm. 49 (2014) 103-106.

[15] L. Li, L. Xu, Z. Li, Y. Bai, H. Liu, Novel nanomaterials used for sample preparation for protein analysis, Anal. Bioanal. Chem. 406 (2014) 35-47.

[16] Y. Yan, S. Ding, D. Zhao, R. Yuan, Y. Zhang, W. Cheng, Direct ultrasensitive electrochemical biosensing of pathogenic DNA using homogeneous target-initiated transcription amplification, Sci. Rep. 6 (2016) DOI: 10.1038/srep18810.

[17] R. Agoston, E. Izake, S. Arumugam, W. Lott, M. Sillence, R. Steel, Rapid isolation and detection of erythropoietin in blood plasma by magnetic core gold nanoparticles and portable Raman spectroscopy, Nanomedicine 12 (2016) 633-641.

[18] J.W. Perez, E.A. Vargis, P.K. Russ, E.R. Haselton, D.W. Wright, Detection of respiratory syncytial virus using nanoparticle amplified immuno-polymerase chain reaction, Anal. Biochem. 410 (2011) 141-148.

[19] C.J. van Oss, R.J. Good, M.K. Chaudhury, Nature of the antigen-antibody interaction. Primary and secondary bonds: optimal conditions for association and dissociation, J. Chromatogr. 376 (1986) 111-119.

[20] W.A. Hassanain, E.L. Izake, M. Schmidt, G.A. Ayoko, Gold nanomaterials for the selective capturing and SERS diagnosis of toxins in aqueous and biological fluids, Biosens. Bioelectron. 91 (2017) 664-672.

[21] S.R. Narayanan, Preparative affinity chromatography of proteins, J. Chromatogr. A 658 (1994) 237-258.

[22] D. Pirici, L. Mogoanta, S. Kumar-Singh, I. Pirici, C. Margaritescu, C. Simionescu, R. Stanescu, J. Histochem. Cytochem. 57 (2009) 567-575.

Antibody Elution Method for Multiple Immunohistochemistry on Primary Antibodies Raised in the Same Species and of the Same Subtype

[23] P.J. Thurmes, D.P. Steensma, Elevated serum erythropoietin levels in patients with Budd-Chiari syndrome secondary to polycythemia vera: clinical implications for the role of JAK2 mutation analysis, Eur. J. Haematol. 77 (2006) 57-60.

[24] C. Zhu, G. Yang, H. Li, D. Du, Y. Lin, Electrochemical Sensors and Biosensors Based on Nanomaterials and Nanostructures, Anal. Chem. 87 (2015) 230-249.

[25] S. Arumugam, E.L. Izake, R. Agoston, G.A. Ayoko, M. Sillence, Reproducible and label-free biosensor for the selective extraction and rapid detection of proteins in biological fluids, J. nanobiotechnol. 13 (2015) DOI 10.1186/s12951-015-0102-8.

[26] W. Putzbach, N.J. Ronkainen, Immobilization Techniques in the Fabrication of Nanomaterial-Based Electrochemical Biosensors: A Review, Sensors 13 (2013) 4811-4840.

[27] R.G. Pillai, M.D. Braun, M.S. Freund, Electrochemically Assisted Self-Assembly of Alkylthiosulfates and Alkanethiols on Gold: The Role of Gold Oxide Formation and Corrosion, Langmuir 26 (2010) 269-276.

[28] L. Zhang, Y. Wang, J. Wang, J. Shi, K. Deng, W. Fu, rhEPO/EPO discrimination with ultrasensitive electrochemical biosensor based on sandwich-type nano-Au/ZnO sol–gel/nano-Au signal amplification, Biosens. Bioelectro. 50 (2013) 217-223.

[29] B. Tokgöz, C. Utas, A. Doğukan, O. Oymak, F. Keleştimu, Influence of long term erythropoietin therapy on the hypothalamic pituitary thyroid axis in patients undergoing CAPD , Renal Failure 24 (2002) 315–323.

Highlights

- A new electrochemical direct biosensing of proteins in blood is described
- The disulphide bonds of erythropoietin are reduced by chronoamperometry
- The protein reduction led to its assembly on nanostructured gold electrode
- The assembled protein was electrochemically desorbed by DPV.
- rhuEPO was quantified by DPV down to 1 pM without signal amplification.
- The new method is simple, rapid and useful for monitoring EPO doping